

Just Microbiology?

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Dr Peter Silley, Research Director at Don Whitley Scientific and Managing Director of MB Consult Limited, a microbiological business consultancy has significant experience within the microbiology industry. With Degrees from the Universities of Birmingham and Newcastle upon Tyne, Peter has also spent time with Cyanamid, ICI and Glaxo and has had appointments with the Department of Agriculture for Northern Ireland and a lectureship at Queen's University of Belfast. At Glaxo he worked both in veterinary and human medicine before leaving to establish the contract microbiology services division of Don Whitley Scientific. Dr Silley also lectures at the University of Bradford as Treasurer of the Society for Applied Microbiology and an Advisor to the Public Health Laboratory Service as well as serving on numerous other Committees.

Is it possible to run a contract research organisation (CRO) as a focused single science discipline... the answer is yes, if that discipline happens to be microbiology. The proof lies in the success of the Don Whitley Scientific business which has grown dramatically over the last seven years and now works with most of the major multi-national as well as smaller pharmaceutical companies. The success of a single discipline contract house in the complex and rapidly changing world of pharmaceutical development appears to be at odds with the industry trend of mergers and consolidation. Benoit Bouche, CEO of FDM Pharma recently described three distinct sorts of CRO

- Rent-a-body companies
- Specialised service providers
- Drug development specialists

He commented on the intriguing future of the large fully serviced, publicly quoted CROs in which the market leaders command 10 to 20% market share whilst their major clients have little more than 4%, "in no other sector does this anomalous relationship between client and suppliers exist". Where will these CROs go next and will they be able to rapidly respond to the changing needs of industry? It is against such a background that the smaller CROs are able to prove their worth in terms of expertise, flexibility and service. Antimicrobial research has not continued to provide a stream of novel compounds over recent years. The diminishing return on research investment and the corresponding efficacy of established products has resulted in a number of companies downsizing their in-house antimicrobial expertise. Ever alert to opportunity the international microbial population has responded leading to tabloid headlines such as

"Hospitals helpless in killer bug battle"

"Superbug wars"

"Antibiotic misuse breeds diseases"

As we all are aware the international aspects of antibiotic resistance have led to a plethora of meetings to address these issues. In 1995 a US Task Force recognised the emergence of antibiotic resistance as a serious problem and made recommendations about the establishment of a national surveillance system, better education to reduce inappropriate usage and more research to develop new products. WHO continued the momentum and held meetings in October 1997 and in June 1998. In September 1998, European Chief Medical Officers met in Copenhagen and recognised that the major contributor to antibiotic resistance in human pathogens was clinical usage in human medicines.

They further acknowledged that the overall reduction of antibiotic resistance required the pursuit of common principles in both human and veterinary medicine. Why is all this relevant to CROs, well it has clearly placed the focus very much back onto the microbiologist and the need to keep abreast of both scientific and political developments. In what many claim was a politically rather than science motivated move the Council of Agriculture Ministers decided in December 1998 to ban four antibiotics, as growth enhancing agents from 1 July 1999 (Council Regulation (EC) No 2821/98). Such issues have provided opportunities for the small CRO to demonstrate their flexibility and expertise in providing the industry with a first class service. No longer can the industry maintain a clear separation between human and veterinary medicine. Many of the questions we are facing require new thinking and openness of mind. The opportunity to work with different classes of compound as well as with both human and veterinary interests gives the CRO a freshness of approach which was often lacking in a company only having experience of one class of compound and one clinical market.

New techniques using real time analytical systems and molecular approaches are adding extra pieces to the jigsaw of our understanding. To provide the industry with the service and intellectual input which it requires demands that the CRO is at the forefront of the appropriate technologies. A CRO must maintain their own R & D programme to ensure they are working at the sharp edge of technology, if not they are unlikely to be able to give you the added value that you need.

It is also more than ever crucial that CROs are involved at the highest level within the industry and their own discipline. Added value to your strategic R & D programme can only be found from a CRO at the forefront of developments in science and politics on an international stage. As with all organisations even the small CROs will evolve to meet the ever changing challenges. In order to maintain an involvement at the political and business level Don Whitley Scientific separated the increasing volume of consultancy work from the laboratory services side of the business with the formation of a new company MB Consult Limited. This development has provided for an improved service to the client base and will ensure that we maintain and build upon our expertise in microbiology.

There is clearly a niche market for the dedicated microbiology CRO and as interest in microbiological issues shows no signs of diminishing that market will surely grow. Servicing it will remain a challenge yet the small CRO without the inevitable bureaucracy of the large companies will always have an enviable advantage.

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PCR-v-Plates

Molecular Approaches - Another Tool for the Food Microbiologist

Whilst the range of techniques available to the food microbiologist has escalated over recent years the majority of laboratories are still using traditional approaches. These "safe" and well accepted methods often require several days to complete and still may fail to detect important pathogens present in low numbers in the food matrix. The food industry has for many years sought to find alternatives to these traditional methods for detection of pathogens, spoilage organisms and general microbial load and indeed a number of significant advances have been introduced over the years.

Most notable has been the introduction of spiral plating in the late 1970s by Ed Campbell (Gilchrist *et al*, 1977) followed by impedance microbiology in the 1980s. Both technologies clearly still have a role in today's food laboratory and offer substantial benefits with regard to savings in time and cost yet they are both based on cultural microbiology.

The detection of food-borne bacteria by classical plate count techniques is highly discriminatory, effective and inexpensive and has played a significant role in food safety over the years yet it is under attack simply because of the length of time it takes to get a result.

Food-borne pathogens are a major concern of any producer as their presence in a food product can result in substantial costs to their business. Contamination levels will generally be low and as there is a requirement for total absence in the tested sample, detection methods normally require lengthy culture enrichment steps to increase target numbers prior to analysis. Culture enrichment is thus a pre-requisite for pathogen screening. This is true not only for agar plate based technologies but also for the immunological enzyme-linked immunosorbent assays and the nucleic acid-based DNA or RNA hybridization protocols. Whilst these latter mentioned approaches are faster they have to date been limited by relatively high detection limits and so have not been able to completely eliminate culture enrichment.

The introduction of nucleic acid amplification methodologies, most notably polymerase chain reaction (PCR) are coming of age and finding widespread application in microbial detection. A significant challenge with the currently available technology is that it only interrogates a sample for a pre-selected target organism and as such will not detect the presence of pathogens *per se*.

Detection limits for many PCR protocols have remained disappointingly higher than desired and most PCR applications for the detection of food-borne pathogens still require enrichment.

Conventionally PCR is carried out on a sample using primers chosen to amplify a particular target gene sequence within a given organism. When the identity of one or more organisms in a sample is to be established it is necessary to guess what gene sequences or organisms may be present in the sample, identify and obtain specific primers for those organisms and then conduct the PCR.

The late Gordon Stewart in an excellent Colworth Prize Lecture entitled "Challenging food microbiology from a molecular perspective" (Stewart, 1997) clearly stated that the ideal situation would be to have a set of primers that would provide an amplified DNA product from any bacterium in a sample and where some variable feature of the amplified product could give information on the identity of the unknown. This concept has been considered previously with ribosomal DNA primers and will no doubt feature again in this rapidly developing field.

The widespread application of PCR in food microbiology has been limited by a number of factors the first of which is the high sample volumes compared to amplification volumes. Consider for a moment the need to ensure absence of *Salmonella* from a 25 g sample of pork pie. However sophisticated is the amplification procedure it becomes rather difficult to get 25 g of pork pie into a thermocycler!

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A second limitation which is now very well documented is the inhibition of the PCR reaction by residual food components. Wilson (1997) reviewed the inhibition of nucleic acid amplification and showed there to be a range of inhibitory compounds in clinical and food samples which acted in different ways. It is clear that as a better understanding of such inhibition is achieved, improved protocols will become available.

Such protocols are at last emerging from the research laboratory and being translated into products. The BAX system from Qualicon is able to detect and identify Salmonella as is the TaqMan commercially available system from PE Applied Biosystem which uses the 5' Nuclease assay in an automated PCR amplification and detection system. Another PCR based commercial system is Probelia introduced by Sanofi Diagnostics Pasteur which is presented as a kit with a colorimetric endpoint.

Other limitations relate to low levels of contaminating pathogens and also the inability to discriminate between live and dead pathogens.

Many of these protocols could clearly be improved if the target bacteria were able to be easily separated, concentrated and purified from the food matrix prior to detection. Progress in this area will help considerably. Whilst the future is bright for molecular technology we are reminded by Wilson (1997) that although DNA amplification technologies continue to provide useful tools for the detection and investigation of microorganisms, their promise will not be completely fulfilled until improved and automated amplification and detection systems become available at affordable prices. For such technology to be widely applicable, methods which allow the rapid and efficient removal of inhibitors and attenuators of amplification must exist. The food microbiologist must consider molecular based methods as another tool to add to and work alongside established culture based protocols.

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